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EFFECTS OF NANO COPPER USED IN SEED TREATMENT FOR GERMINATION, GROWTH, AND PRODUCTIVITY OF MAIZE

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ABSTRACT

Recently, metal and other nanoparticles have been widely used to improve crop growth and development, reduce used chemical amount, increase safety of agricultural products and prevent soil and water pollution. Seed treatment technology with metal nanoparticles to stimulate seed germination and improve crop yields has been reported in several studies. In order to assess the safety of the use of metal nanoparticles in agriculture production, in this study the effect and safety of seed treatment with nanoparticles (nCu) prior to sowing on the germination, growth, development, productivity and quality of maize (*Zea mays* L.) were evaluated in the field conditions. Seeds of maize cultivar LVN 092 were treated with nCu at different concentrations before planting. Germination and plant growth rates, plants yield and grain nutrition were monitored and evaluated. The obtained results showed that treatment of maize seed with nCu at a concentration of 20 mg/kg seed increased germination after 7 days of sowing as well as the theoretical and actual yields compared to the control and the treatment of 1000 mg nCu/1 kg seeds. Other agricultural characteristics, such as the development rate, plant height, grain nutritional content including moisture, ash, total fiber, crude protein, minerals were not significantly between treatments and the control. Therefore, the seed treatments with metal nanoparticles can be contribute to improve agriculture production without impact to quality of crops.

Keywords: Zea mays, metal nanoparticles, seed treatment, plant growth development.

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INTRODUCTION

Nowadays, nanomaterials have been wildly used in agriculture as fertilizers, growth regulators, plant protection agents to stimulate crop productivities due to their particularly small size and unique properties (Roduner, 2006; Adhikari et al., 2010). A number of studies focused on the effectiveness and safety of using metal nanoparticles in agriculture production (Alloway, 2008; Sheykhbaglou et al., 2010; Rico et al., 2011; Kole et al., 2013; Begum et al., 2014). Among the micronutritinal elements required for normal plant development, manganese (Mn), copper (Cu), iron (Fe), zinc (Zn), boron (Bo), chlorine (Cl), molybdenum (Mo), and cobalt (Co) are essential for the growth of plants (Alloway, 2008). The providing of these substances to plants directly through leaves or indirectly to the soil had contributed to the plant development and the fertility of the soil (Peteu et al., 2010). Bitter melon (*Momordica charantia*) seed treatment with fullerol ($C_{60}(OH)_{20}$) increased the yield, fruit length, fruit number/plant and fruit weigth up to 54, 20, 59 and 70%, respectively. The content of the two anticancer agents cucurbitacin-B and lycopene in fruit incrased 74 and 82%, respectively. The content of charantin and insulin, two active substances to treat diabetes, also increased by 20 and 91% (Kole et al., 2013). Sheykhbaglou et al. (2010) conducted the the experiment on the effect of FeO nanoparticles on the yield of soybean (*Glycine max*) grown in the field and found that at the concentration of $0.5 g/dm^3$, FeO nanoparticles helped to increase yield to the impressed level, reaching 48% compared to the control.

There are also several studies to evaluate the undesirable effects of nanomaterials on plants and indicated that the majority of nanoparticles were beneficial or have no significant effect on plant growth; adversely effects on certain crops were reported in limited casses (Rico et al., 2011; Begum et al., 2014). When evaluating the possible toxicity of the five nanoparticles, Lin & Xing (2007) found that only Zn and ZnO nanoparticles inhibited germination and root development in six plant species. Similarly, when seed of maize, alfalfa (*Medicago sativa*), cucumber (*Cucumis sativus*) and tomato (*Lycopersicon esculentum*) treated with nano ceria (Ce) at concentrations from 0 to 4000 mg/L, germinaton cability of maize, tomato and cucumber decreased 30, 30 and 20%, respectively, compared to the control at 2000 mg/L dose use. While roots of maize and cucumber were stimulated, they were decreased for alfalfa and tomato. Most of the nano-Ce concentrations boosted sprouting in all four species (Lospez-Moreno et al., 2010). The development of *Cucurbita pepo* (Cucurbitaceae) was decreaed by Ag and Cu nanoparticles (Musante & White, 2010). Very few studies have been conducted to evaluate the accumulation of nanoparticles in plant species and the presence of these materials in food crops. However, it can be seen that most of reported studies were carried out under conditions of high concentrations of nanoparticles used.

In Vietnam, the nanomaterials have recently been used as fertilizer or plant growth regulatory of many cultivation species. Several studies have been conducted to evaluate the role of Fe, Co and Cu metal nanoparticles in the growth and development of soybean, maize and vegetables. Bui (2009) had produced silver nanoparticles (Ag) by gamma Co-60 radiation to treat fungal blast disease (*Piricularia oryzae* Cavara), grain rot disease (*Pseudomonas glumae* Kurita et Tabei). Pesticides, which are effective to manage pink fungus *Corticium salmonicolor* and white powdery mildew *Oidium heveae* of rubber trees, were produced from Cu nano solution by Nguyen et al. (2011). Using sol-gel method, Dong et al. (2013) prepared a white colour and dispersibility TiO_2 solution to inhibit the growth and symptoms of tobacco mosaic virus (TMV) in plants. Duong et al. (2015) studied the *in vitro* growth and development of *Chrysanthemum* sp. in medium supplimented with nFe. The obtained results indicated that the growth of young shoots was excessed compared to the controls, leaves stayed green and root formation was faster in the presence of nFe and nFe-EDTA. In addition, nAg also had a positive effect on the growth and development of strawberry (*Fragaria* sp.) and gerbera (*Gerbera* sp.) cultured *in vitro* (Duong et al., 2014). They also demonstrated the effect of Ag nano on the growth of *Chrysanthemum morpholium* in hydroponic systems. The plants were speeded after 2 weeks in the medium added with 7.5 ppm nAg. The present of nAg also inhibited the growth and development of 8 bacterial and 3 fungal species. In addition, such *C. morpholium* plants gave a higher survival rate (100%) and better growth than other treatments when transferring to the green house (Hoang et al., 2016). For maize, Fe, Co, and Cu nanoparticles were reported to increase maize productivity (Churilov et al., 2012; Ngo et al., 2013, 2014).

This study aims to assest the safe use of nanometal particles, and the effect of Cu nanoparticles (nCu) at the optimal and 500 fold higher concentrations was monitored and evaluated. The results contributed to the safe application of nanomaterials to increase productivity and quality of agricultural products.

MATERIALS AND METHODS

Hybrid maize cultivar LVN 092, which was characteried by low ear insertion, strong sterm and root system, was provided by the Maize Research Institute. Shiny, uniform in size and free from damage seeds were carefully selected for this study.

Cu nanoparticles are provided by the Institute of Environmental Technology, Vietnam Academy of Science and Technology within the framework of the Project: "Application of nanotechnology in agriculture". Seeds were treated with nCu at three different concentrations of nCu: (i) optimal concentration of 20 mg/1kg seed (F1) and (ii) 1000 mg/1 kg seed (F2); (iii) the control seeds were wetted with water (Control). Solution of nCu was prepared by dispersing the metal nanoparticles in water and placed in ultrasound (800 W, 20 kHz) for 30 minutes. Next, the selected seeds (1 kg) were mixed with the prepared nCu solution, incubated for 45 minutes, removed from solution and dried in the air for 1–2 hours before sowing (Churilov et al., 2000; Churilov, 2010).

Cultivation

After nCu treatments, seeds were dried and sown in 9 expreriment plots. Each plot consisted of 7 rows of 10 m in length, a distance of row and plant in a row to be 70 × 25 cm, corresponding to 49 m^2/plot. Cultivation and fertilization methods were done in accordance with Trial Regulations 01-56-2011 of Ministry of Agriculture and Rural Development. The experiments were conducted in the Summer-Autumn cultivation season of 2016 at the Maize Research Institute, Vietnam Academy of Agricultural Sciences (Dan Phuong, Ha Noi).

Germination assay

Germination percentage (GR) and seedling establishement (SE) were monitored and culculated after 7 days, in which GR = [Germinated seeds/total]×10%; SE= [Number of formed seedling/total] × 100 (%).

Growth and development of maize plants

These morphological characteristics were evaluated via plant height (measured from the ground to the leaf end) in centimeter at 30, 40 and 50 days after planting.

Theoretical and actual yields

In each plot, five plants in two random rows were measured. Theoretical and actual yield (weight/ha) are calculated according to the following formulars:

TY (Theoretical yield) = No of plants/m^2 × No of ears/plant × No of row/ear × No of grains/No of rows of grain × 1000 grain weight/10.000

AY (Actual yield) = $(P_1/S_0) \times (P_2/P_3) \times (100-A_0)/(100-14) \times 10^2\ m^2$

In which: P_1: fresh ear weight; A_0: grain moisture; S_0: plot acreage; P_2: grain weight of samples (weighted at the time of measuring the seed moisture content "A_0"); P_3: fresh seed weight.

Nutrient analysis

Water content (%) was determined by the Benjamin and Grabe methods (1988). Specifically, 10 g of grains was placed in a porcelain bowl and dried at 105°C for at least 6 hours to reach a constant weight. Following, the porcelain cup was cooled in a desiccator for about 25 to 30 minutes and then weighed with analytical balance (accuracy of 0.0001 g). Water content was calculated according to the formular: Water content (%) = [(m_1-m_2)/ (m_1-m)] × 100 (%); in which m, m_1 and m_2 are the weights of the porcelain cup, porcelain cup containing 10g sample before and after dried at 105°C, respectively.

The ash content was determined using the high heat (550–600°C) to completely burn organic substances (AOAC, 1999). A total of 5 g of the sample was placed into the cup and baked in a oven at 600°C for 6 to 7 hours until ash having white color. The porcelain cup was cooled in a desicator and weighed with an accuracy 0.0001g. Ash contain was measured as the following fomular: Ash content (%) = [(m_2-m)/ (m_1-m)] ×100 (%), in which m, m_1 and m_2 are the weights of the porcelain cup, porcelain cup containing 5g sample before and after heated at 600°C, respectively.

Total dietary fiber (TDF) was determined by enzyme-mass method according to TCVN 9050:2012. Seeds were hydrolyzed by heat-stable α-amylase, protease and amyloglucosidase to remove starch and protein. Subsequently, the soluble fiber in the hydrolyzed solution was precipitated with ethanol before filtration and the total fiber residue was washed with ethanol and acetone, dried at 105°C, cooled in a desiccator and weighed using a balance with accuracy at 0.0001 g. Total dietary fiber was determined as following formula: TDF (%) = [(precipitated pellet weight-ash weight-protein weight)/ initial sample weight] × 100 (%).

Crude protein content: The crude protein content was determined using Kjeldahl method indicated in TCVN 10791:2015. Total 1 g of the sample was added into digestion vessel with 10 g of potassium sulphate, 0.7 g of mercury oxide and 20 mL of concentrated sulfuric acid. The mixture was heated slowly until foam appearance, boiling and becoming transparent. The solution was then cooled and added with 90 mL distilled water and then 80 mL of NaOH 2M to form two layers in the extractor. Condensed ammonia was collected into a tube containing 50 mL boric acid and methyl red indicator. The condensate (50 mL) was recovered and titrated by HCl 0.1M. The percentage of nitrogen was assessed as follows: Nitrogen (%) = [Acid volume × No of mol of standard acid]/[Sample weight] × 0,014 × 100 (%); Crude protein content (%) = Nitrogen content × 6.25.

Determination of mineral elements in maize grain: Mineral elements were analyzed by using Van loon method (1996). One gram of crushed sample was placed in a 250 mL conical flask, and then added 15 mL of HNO_3 and 5 mL of concentrated H_2SO_4. The solution was well mixed and heated on a stable stove at a temperature of 160°C until brown color disappears and white gas appears. Total 10 mL H_2O_2 was added to the mixture and continued to heat until dry. The hydrolyzed sample was cooled and the residue was dissolved slowly with deionized water until reaching solution volume of 100 ml. The mineral elements were determined on atomic adsorption spectrophotometers.

Data analysis

Each experiment was done with 3 replicates and was expressed as the mean value ± standard deviation. Data were collected and compared using Student *t-test* with significant difference at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of nCu treatment on germination of maize seeds

After seeds treated with nCu at the optimal and high concentrations, germinated rate were 96.8% for F1 and 97.2% for F2, which means a non-significant result between the treaments and the control (100%, $P > 0.05$). The germination seed continued to be monitored for germination power, the ability of seeds to grow uniformly after 7 days in the experimental plots. Although the germination rate was similar among treated and control, the germination power of the seeds at F1 was higher than the seeds at F2 and control (96, 90.07 and 90.03%, respectively) ($P < 0.05$) (Fig. 1).

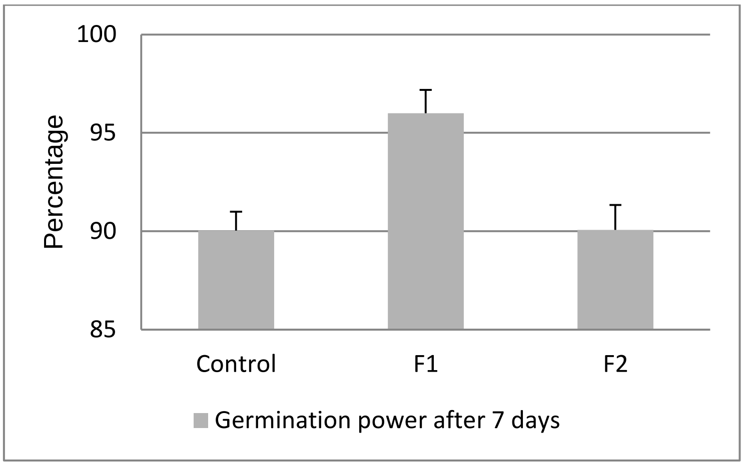

Figure 1. Germination power of nCu treated LVN 092 seeds

Several studies have been carried out to assess the effect of nanomaterials on cultivated species. Toxicity assesment of 5 nanoparticles (multi-walled carbon nanotubes, aluminum, aluminum compounds, Zn and ZnO) showed that only Zn and ZnO nanoparticles inhibited the germination and growing roots of grass and maize plants, respectively, when the concentration of 2000 mg/L was used to treat seeds (Lin & Xing, 2007, 2008). Musante White (2010) discovered Ag and Cu nanoparticles to reduce the development of pumpkin plant (*Cucurbita pepo*). Examinating the effect of TiO_2 and Fe_3O_4 nanoparticles on tomato plants cultivated in hydroponic system, Giordani et al. (2012) observed the number of root hair of seedlings grown in medium containing 500 mg/L TiO_2 nanoparticles was significant higher than the control plants or the one treated with 50 mg/L and 500 mg/L Fe_3O_4 nanoparticles; additionally, the treated plants with nanoparticles were not morphologically changed because the toxicity were undetected in plants. Similarly, in our study, nCu at low and 500 fold higher concentrations did not affect the germination ability of maize seed LVN 092. At the optimum concentration, nCu even promoted the germination power.

Evaluate the growth and development of maize plants from nCu treated seeds

The seedling vigor was uniform among the experimental plots after 14 days planting. The analysis of plant devolopment at 30, 40 and 50 days after growing showed that the plant height in 3 experimental plots did not differ significantly ($P > 0.05$). Growth rate in the period of 30–40 days was fater compared to the period of 40–50 days and the same for 3 experiment plots during the studied period ($P > 0.05$) (Table 1). This suggests that nCu could support seed germination without affecting the plant height as well as plant growth. It also is worth to note that although seeds treated with nCu at different concentrations, the plants still grow normally as the control together with uniformity morphology in whole develpment period.

Table 1. Plant growth and development characteristics of nCu treated seeds

	Control	**F1**	**F2**
Plant height (cm)			
30th day	66,27 ± 8,07	73,20 ± 6,40	60,03 ± 6,72
40th day	138,27 ± 9,15	139,97 ± 4,91	139,80 ± 7,20
50th day	171,20 ± 9,62	176,80 ± 7,86	172,50 ± 3,10
Growth rate (cm/day)			
From 30th to 40th day	7,20 ± 1,25	6,68 ± 0,55	7,98 ± 0,85
From 40th to 50th day	3,29 ± 1,36	3,69 ± 0,96	3,27 ± 0,73

Analysis of theoretical and actual yields

Crop productivity is the most important factor for a cultivared crop species. Breeding, fertilizer application, or intensive farming are all aimed at improving productivity. In this study, statistical analysis showed that the theoretical and actual yields in F1 using seeds treated at optimal concentration of nCu was higher than that of the control ($P < 0.05$). Above result could prove that nCu at an optimal concentration not only stimulate seed germination, but also have a positive effect on plant productivity. In addtion, at high concentration of 1000 mg/kg, the yield from treated seeds was not different from that of control. The obtained results of this study are consistent with many other studies concerning the impact of nanoparticles on plant species. Sheykhbaglou et al. (2010) testing the effect of FeO nanoparticles at concentrations of 0.75 g/L and 0.5 g/L on agronomic traits of field-grown soybean found that at concentration of 0.5 g/L, it increased yield to the highest level of 48% compared to the control. Other studies demonstrated that low concentrations (20 mg/L) of TiO_2 nanoparticles promoted photosynthesis and nitrogen metabolism (Hong et al., 2005a, b; Yang et al., 2006) as well as mRNA replication and protein expression in plant cells leading to increasing the growth of spinach (Gao et al., 2006). It is also noteworthy that the mixture of SiO_2-TiO_2 nanomaterials had the ability to increase reductase nitrate enzyme in soybeans, help the plants enhancing the absorption and use of water and fertilizer, stimulating the antioxidant system, germination and growth (Lu et al., 2002). Studies on the transport mechanism and expression level of metal binding genes in germinated rice seeds treated with Zn, Fe, Cu and Mn nanoparticles indicated that the nanoparticles were transported from the culture medium into the endosperm (part containing starch) of the rice grain, then dispersed to the secondary roots, leaves meristem during whole germination stages of the rice grain via the expression of intermediates nicotianamine (NA) and deoxymugineic acid (DMA) in the biosynthetic pathway of the mugineic acid (MAs) group - phytosiderophores, which plays an important role in the transport and accumulation of metals in grain development process (Takahashi et al., 2009). Recently, Giraldo et al. (2014) announced 3 fold increased in photosynthesis on chloroplasts of *Arabidopsis* treated with carbon nanotube material encasulated by single strand of DNA. Churilov et al. (2012) and Ngo et al. (2013; 2014) evaluated the effect of metal nanoparticles on the germination and growth of maize and reported that germination rate, leaf surface area, leaf weight, root weight, root length and plant height incresed by 14; 22.2; 25; 27.3; 28.3 and 17.2%, respectively. In addtion, Fe, Co and Cu nanoparticles promote both yield and quality of harvested products. Salama (2012) demonstrated a positive effect of silver nanoparticles on the growth of several crops, including bean (*Phaseolus vulgaris* L.) and maize. Addition of Ag nanoparticles at concentrations of 20 to

60 ppm increased the length of shoots and roots, leaf surface area, chlorophyll, carbohydrate and protein contents of beans and maize.

Table 2. Effect of nCu on productivity of LVN 092 maize

Traits	Control	F1	F2
Weight of 5 ears (kg)	0,723 ± 0,025	0,843 ± 0,021	0,777 ± 0,051
Seed weight/ 5 ears (kg)	0,587 ± 0,015	0,697 ± 0,025	0,620 ± 0,046
Fresh seed moisture	24,767 ± 1,026	24,167 ± 0,153	25,300 ± 0,200
Theoretical yield (0.1 ton/ha)	48,683 ± 0,607	58,080 ± 1,482	51,523 ± 1,865
Actual yields (0.1 ton/ha)	20,756 ± 0,259	32,026 ± 0,817	21,902 ± 1,709

Analysis of nutrient content of harvested maize grains

To assess the effect of nCu on nutrient value, maize grain in each experimental plot was harvested. Moisture, ash, crude fiber and total crude protein contents of seeds were analyzed. The results show that there was no difference of all interested sustances among exprimental plots (Table 3, Fig. 2).

Moisture is the amount of free water in the grain. Small changes in water content may greatly affect the storage life and germination of seeds (Ali et al., 2014). High water content coupled with the presence of oxygen were the main causes of lipid toxicity in seeds leading to rapid quality degradation of storade seeds (Chang, 2004). The suitable water content of maize grain at 25°C and relative humidity of 30% was 8.4% (Cromarty et al., 1982). In this study, the seed water content in F1 and F2 was not significant diference compared to 8.4% of the control ($p > 0.05$) and therefore it means nCu do not inflluent to water content of grain and the seeds obtained from plants grown using nCu streated seeds maintain as normal seeds.

Ash is the rest of the grain after its organic sustances totally burned. The elements C, H, O, N are lost in the form of CO_2, steam, NO_2, O_2 or N_2. The ash contains only mineral salts. The results in table 3 showed that the ash content of maize grains in 3 plots did not differ from each other and valued from 1.4 to 3.3% as indicated by Maziya-Dixon et al. (2000).

Fiber in grain consists of 2 parts: (i) soluble fibers including some special compounds called prebiotics including gum, mucilage, pectin are short-chain polysaccharide and can be soluble in water; and (ii) insoluble fibers are long-chain polysaccharides and water-insoluble. In this study, the total fiber content was quite low in all 3 experimental lots.

The crude protein content of corn grain ranges from 9.23 to 9.85%. According to statistical analysis, the crude protein content of grain of F1 and F2 was not different from that of maize in the control plot ($p > 0.05$). This indicates that using nCu to treat seeds before sowing did not affect seed quality.

In addition, storage mineral element in seeds have a high biological value to humans, and are essential for grain entering the early stages of germination and growth, direct involvement in building living cells, regulating growth and development in plants. Corn seeds from experimental treatments were analyzed for the content of minerals K, Ca, Mg, Fe and Zn. Results showed that accumulation levels of mineral elements were safe and intoxicated to plants or health of users. The content of K was the highest, followed by Mg, Ca, Fe, and Zn. These results are consistent with the report of Gwirtz & Garcia-Casal (2014).

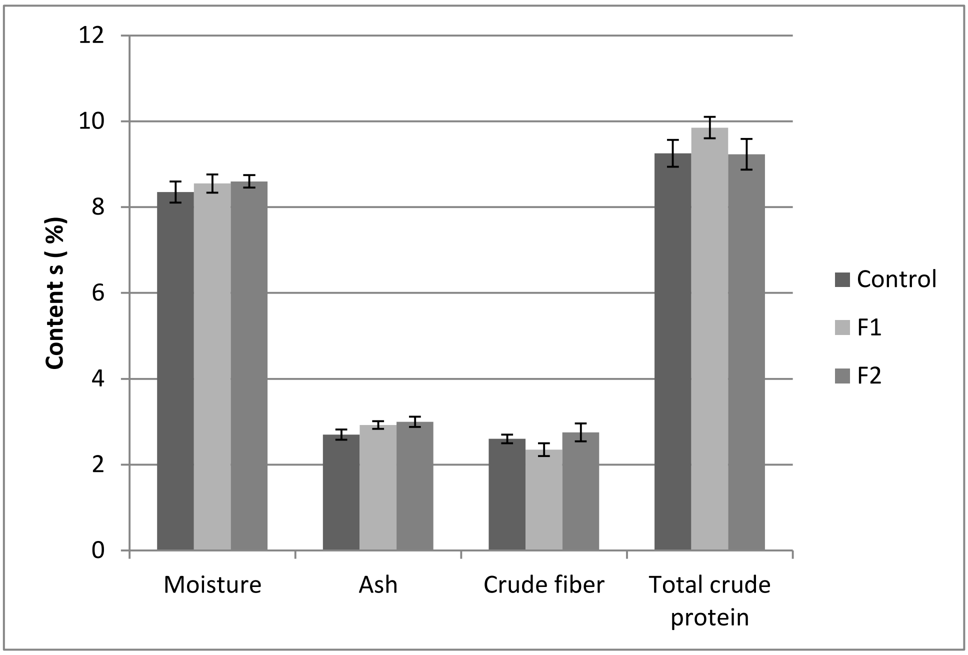

Figure 2. Nutritional quality of grain

CONCLUSION

Using nCu at low concentration of 20 mg/kg for treatment of maize seeds before planting promoted germination rate, germination power, plant growth and development, that increased productivity and levels of mineral elements compared to the control. Using nCu of high concentration (e.g. 1000 mg/kg of seeds) also caused a non-significant effect on germination rate, plant growth and development, and nutrient values of the treated seeds.

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